PB82-127390

Fluorescence Depolarization Studies of Red Cell Membrane Fluidity. The Effect of Exposure to 1.0-GHZ Microwave Radiation

(U.S.) Health Effects Research Lab.
Research Triangle Park, NC

Sep 81

U.S. Department of Commerce
National Technical Information Service
NTIS.

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA-600/J-80-137	2. JOURNAL ARTICLE	3. REC PB82-127390
4. TITLE AND SUBTITLE FLUORESCENCE DEPOLARIZATION STUDIES OF RED CELL MEMBRANE FLUIDITY. THE EFFECT OF EXPOSURE TO 1.0-GHZ MICROWAVE RADIATION		5. REPORT DATE September 1981 6. PERFORMING ORGANIZATION CODE 308049
7. AUTHOR(S) JOHN W. ALLIS AND BARBARA L. SINHA		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Experimental Biology Division Health Effects Research Laboratory US Environmental Protection Agency Research Triangle Park, NC 27711		10. PROGRAM ELEMENT NO. ABZE1A 11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS Office of Research and Development (RTP,NC) Health Effects Research Laboratory US Environmental Protection Agency Research Triangle Park, North Carolina 27711		13. TYPE OF REPORT AND PERIOD COVERED 14. SPONSORING AGENCY CODE EPA-600/11

15. SUPPLEMENTARY NOTES

Published In: Bioelectromagnetics 2:13-22, 1981

16. ABSTRACT

The internal viscosity of human red blood cell membrances was investigated during exposure to continuous wave 1.0-GHz microwave radiation using fluorescence measurements of a lipid seeking molecular probe, diphenylhexatriene. Samples were exposed in a Crowford cell arranged so that fluorescence was measured during microwave exposure; specific absorption rates calculated from electrical measurements were approximately 0.6, 2 and 15W/kg. Measurements were obtained at selected temperatures between 15°C and 40°C and as a function of the duration of exposure at 23°C. Arrheniustype plots of the temperature profile data were linear and showed no difference between exposed and control samples. The exposure duration data also showed no difference between exposed and control samples except for a small effect of elevated temperature at the highest exposure. The activation energy for motion of the fluorescent probe in its environment within the membrane lipid was not affected by the application of the microwave energy and no evidence for a lipid phase transition was found. These results indicate that the increased cation efflux from red cells, observed by others at certain transition temperatures during microwave exposure, was more likely to have been caused by alteration of the membrane bound protein than by changes in the lipid constituents of the red cell membrane.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA 22161

18. DISTRIBUTION STATEMENT RELEASE TO PUBLIC	19. SECURITY CLASS (This Report) UNCLASSIFIED	21. NO. OF PAGES
	20. SECURITY CLASS (This page) UNCLASSIFIED	22. PRICE

EPA-600/J-80-137
Journal Article

Bioelectromagnetics 2:13-22 (1981)

Fluorescence Depolarization Studies of Red Cell Membrane Fluidity. The Effect of Exposure to 1.0-GHz Microwave Radiation

John W. Allis and Barbara L. Sinha

Health Effects Research Laboratory, US Environmental Protection Agency, Research Triangle Park, North Carolina

The internal viscosity of human red blood cell membranes was investigated during exposure to continuous wave 1.0-GHz microwave radiation using fluorescence measurements of a lipid seeking molecular probe, diphenylhexatriene. Samples were exposed in a Crawford cell arranged so that fluorescence was measured during microwave exposure; specific absorption rates calculated from electrical measurements were approximately 0.6, 2 and 15 W/kg. Measurements were obtained at selected temperatures between 15 °C and 40 °C and as a function of the duration of exposure at 23 °C. Arrhenius-type plots of the temperature profile data were linear and showed no difference between exposed and control samples. The exposure duration data also showed no difference between exposed and control samples except for a small effect of elevated temperature at the highest exposure. The activation energy for motion of the fluorescent probe in its environment within the membrane lipid was not affected by the application of the microwave energy and no evidence for a lipid phase transition was found. These results indicate that the increased cation efflux from red cells, observed by others at certain transition temperatures during microwave exposure, was more likely to have been caused by alteration of the membrane bound protein than by changes in the lipid constituents of the red cell membrane.

Key words: Crawford cell, diphenylhexatriene, fluorescence depolarization, microviscosity, 1-GHz microwave radiation, red cell membranes, temperature dependence

INTRODUCTION

Biological membranes may be affected by exposure to nonionizing electromagnetic radiation, as suggested by several workers [Seaman and Wachtel, 1978; Barnes and Hu, 1977; Keilman, 1978; Frolich, 1968; and Grodsky, 1976]. It should therefore be possible to detect some change in physical or chemical properties of the membrane due to exposure to microwave radiation. Functional properties of a biological membrane are thought to be influenced by changes in the internal viscosity or fluidity of the lipid portion of a membrane [Quinn, 1976]. The fluidity of membrane lipid is temperature dependent, increasing

Received for review August 27, 1980; revision received November 17, 1980.

Address reprint requests to Dr. John W. Allis (MD-74), Experimental Biology Division, Health Effects Research Laboratory, Environmental Protection Agency, Research Triangle Park, NC 27711.

as temperature increases. This phenomenon may be envisioned as an enhanced ability of the hydrocarbon tails of the phospholipids to move within their environment in the membrane. Most membrane lipid species are dipolar; therefore it is reasonable to expect that microwave radiation might alter their molecular motion in a way that would change the temperature profile of the membrane fluidity.

Several workers who have studied the effect of microwave radiation on red blood cells indicate possible effects on the membrane. Baranski et al [1974], Shtemler [1973] and Ismialov [1971] have all found that radiation in the 1 to 3-GHz range affects ion transport across the membranes. Others [Hamrick and Zinkle, 1975; Peterson, et al, 1979; Liu and Cleary, 1979] attempted to replicate Baranski's experiment, but were unable to observe similar effects. More recently Olcerst et al [1980] found increased passive cation transport in rabbit erythrocytes at certain temperatures during exposure to 2.45-GHz radiation. The authors suggest either lipid phase transitions or membrane protein conformational transitions as mechanisms for their effect.

In this paper, we report studies of the internal viscosity of human red blood cell membranes as a function of temperature during exposure to microwave radiation. The fluorescent probe molecule, 1,6-diphenyl-1,3,5-hexatriene (DPH), first introduced by Shinitzky and Barenholz [1974], has been used to monitor viscosity within the hydrophobic portion of the membrane. A fluorometer has been mated to a rectangular transmission line (Crawford cell) so that measurements can be conducted while the sample undergoes continuous exposure at 1.0/GHz. The samples are maintained under controlled temperature conditions so that the fluorescence response of the preparations can be recorded over a series of temperatures during exposure. The dependence on the duration of exposure has also been monitored at one temperature, 23 °C.

MATERIALS AND METHODS

Fluorescent Probe

The fluorescent probe 1,6-diphenyl–1,3,5-hexatriene (DPH) was dissolved in tetrahydrofuran (both obtained from Aldrich Chemical Co.) to give a concentration of 2×10^{-3}M. For each day's experiment fresh probe solution was diluted 1:2000 with hypotonic sodium phosphate buffer (20 mM, pH 7.4) to give a stock solution 1×10^{-6}M in DPH.

Red Blood Cell Membranes

Membranes were prepared from outdated human blood obtained from the blood bank at Duke University Medical Center. Preparation procedures followed closely those of Dodge et al [1963] except that the final wash step was repeated until all traces of pink disappeared. The membranes were then suspended in the hypotonic sodium phosphate buffer at a concentration of 2.5–3.0 mg protein per ml [Murphy & Kies, 1960], and were used within 24 hours. A single unit of blood was used for 2 weeks and discarded. All procedures and storage were at 3 °C.

The fluorescent probe, DPH, was introduced into the red cell membranes on the day of the experiment by mixing equal volumes of the 1×10^{-6}M DPH solution and a 1:12 dilution (hypotonic phosphate buffer) of the membrane suspension. The resulting mixture was incubated at 37 °C for 90 minutes to ensure penetration of the RBC membrane by the probe.

Non-probe-containing suspensions were prepared by substituting hypotonic phosphate buffer for the DPH solution. This suspension was also incubated at 37 °C for 90 minutes and used as the light scattering blank for the fluorescence measurements.

Fluorescence Measurements

Fluorescence depolarization of DPH was measured by a method similar to that described by Shinitzky and Barenholz [1974] using a Perkin Elmer MPF-44 spectrofluorometer. Fluorescence intensity was measured in planes parallel and perpendicular to that of the exciting light. The depolarization data for DPH were obtained using an excitation wavelength of 360 nm and an emission wavelength of 425 nm. The measurements were converted to apparent microviscosity of the lipid portion of the membrane using a method described by Lentz et al [1976], which is based on the theory of fluorescent probe depolarization applied to membrane systems [Shinitzky et al, 1971]. One set of measurements was conducted as a function of temperature and another set was conducted as a function of duration of exposure at a fixed temperature (23 °C).

Two pair of samples were used for fluorescence measurements. Each pair consisted of a cuvette containing the membrane suspension with DPH added and another cuvette containing the membrane suspension alone. Exactly 3.5 ml were measured into each cuvette. One pair was exposed to the microwave radiation and the second pair was the unexposed control. The exposed pair was placed in the fluorometer-exposure system and both cuvettes were exposed until temperature equilibrium was attained. The control pair was placed in a temperature regulated water bath during this time. After depolarization measurements were performed on the exposed pair, the microwave radiation was turned off and the control pair was placed in the instrument long enough to perform the depolarization measurement. The cuvettes were then returned to their original places and the exposure was resumed in preparation for the next measurement. For each measurement the sample fluorescence was corrected for the light scattering contribution of the membranes by subtracting the value of the blank from the corresponding value of the sample.

Microwave Equipment and Dose Measurement

The microwave exposure equipment, which is described in detail elsewhere [Allis and Weil, in preparation], is based upon a small Crawford cell (regular transmission line) mounted vertically at the front of the fluorometer. Special optics were designed so that fluorescence measurements could be performed on a sample placed in the Crawford cell. The membrane suspensions (with and without DPH) were placed in the Crawford cell together, one on each side of the center conductor. The long axis of the sample cuvette was parallel to the center conductor with the top of the cuvette facing the source of the power. The electric vector was perpendicular to the long axis of the cuvette and the magnetic vector was orthogonal to both the electric vector and the long axis of the cuvette. The cuvettes occupied the center two thirds of the distance from the ground plane and center conductor and were not in contact with either.

The microwave field was generated by a Wavetek Sweep Signal Generator (Model 2000, Indiana Inc) set at 1000 MHz, continuous wave. The signal passed through a solid state amplifier (Model LWA 510–20, Microwave Power Devices Inc, 500–1000 MHz), then through a dual directional coupler (Model 3020A, Narda) and into the Crawford cell. Incident and reflected power were monitored at the dual directional coupler and transmitted power was monitored at the lower end of the Crawford cell through a 20-dB attenuator using three power meters (Model 435A, Hewlett Packard).

The rate of energy absorption by the sample and blank was determined from readings of incident, transmitted and reflected power with the exposed pair of cuvettes in place and from similar measurements made on the empty Crawford cell. The average specific absorption rate (SAR) was then calculated for each exposure using the method described by Joines et al [1976].

Temperature Measurement and Control

Temperature of the exposed membrane suspensions was maintained by circulating water from a constant temperature bath through thin-walled Tygon® tubing in contact with the metal walls of the Crawford cell, so that only the surrounding air formed thermal contact with the sample cuvette. Temperature of the exposed samples was maintained within ± 0.2 °C (for an individual fluorescence measurement) and was adjustable over the range 15–40 °C. The control pair was maintained in a water bath at the same temperature as the exposed pair in the Crawford cell. Temperature measurements of the exposed and control samples were taken periodically in the Crawford cell using a telethermometer (Yellow Springs Instrument Co, Model 46 TUC) and a non-metallic thermistor probe (Yellow Springs Instrument Co, Model 520). This probe has been found to interact only slightly with the microwave field [Allis et al, 1977]; however, neither fluorescence measurements nor dose measurements were made with the probe in the microwave field. A Vitek® probe became available to us after the conclusion of these experiments. Using this probe, temperature was measured at 15 locations within the sample at an SAR of 15 W/kg. The temperatures were within 0.1 °C except at the top and bottom of the sample column where variations of 0.2–0.3 °C were often found. These measurements imply that energy deposition is reasonably uniform in the center portion of the sample where the fluorescence measurements are taken.

RESULTS

The rotational motion of the probe, DPH, in the lipid portion of the membrane is dependent upon the degree to which its surroundings allow movement. Therefore the motion of the probe is a direct measure of the local viscosity of the membrane interior. The motion of the probe is characterized by the anisotropy parameter, r, which is obtained from measurements of fluorescence intensity polarized both parallel and perpendicular to the plane of the exciting light:

$$r = (I_{||} - I_{\perp})/(I_{||} + 2I_{\perp}) \quad (1)$$

The apparent microviscosity indicated by the probe molecule is calculated from the anisotropy by means of a modified version of the Perrin equation [see Lentz et al, 1976 for a more complete discussion]. Following the development of Shinitzky and Barenholz [1978], a simplified version of this equation may be obtained for DPH:

$$\bar{\eta} = 2.4r/(r_0 - r) \quad (2)$$

where $\bar{\eta}$ is the microviscosity and r_0 is the limiting value of the anisotropy, equal to 0.362 for DPH.

TABLE 1. Characterization of Red Cell Membranes Exposed to 1.0-GHz Microwave Radiation Slopes of Plots of Log Microviscosity vs 10^3/Temperature*

SAR (W/kg)	Experiment number	Slope		P*	F(1,33)[a]
		Exposed	Control		
0.1	1	1.06	1.10	N.S.	0.47
	2	1.27	1.14		
	3	1.23	1.19		
	4	1.23	1.17		
2.0	1	1.02	1.22	0.05	4.12
	2	1.26	1.36		
	3	1.18	1.30		
	4	1.10	1.16		
2.2	1	1.16	1.23	N.S.	0.00
	2	1.42	1.26		
	3	1.30	1.21		
	4	1.27	1.45		
15.4	1	1.25	1.16	N.S.	0.56
	2	1.19	1.22		
	3	1.25	1.14		
	4	1.34	1.40		

* K

[a] Analysis of variance, see text.

The logarithm of the viscosity of a homogeneous medium is normally a linear function of the inverse absolute temperature (an Arrhenius-type plot). The temperature response of membrane lipid viscosity may also be characterized in this way, but since the membrane is not a homogeneous medium, the information obtained may only apply to the microviscosity in the immediate neighborhood of the probe molecule. Figure 1 represents the temperature profile of the microviscosity of the samples irradiated at 1.0 GHz at three exposure levels and the corresponding controls. As can be seen in Figure 1, the data agree with the linear model. The slope of the line was obtained in each case from a least squares fit to the data. The slopes from each set of measurements are tabulated in Table 1 for each of the three exposure levels. The specific absorption rate (SAR) was measured for each exposure level and the data is presented as a function of SAR in Table 1.

The statistical design of the experiment required that measurements of exposed and control samples be performed on the same day. This was necessary because preliminary results indicated measureable variability in assays performed on different days although the membranes were prepared from the same unit of blood. Four pair of measurements were performed on membranes prepared from a single unit of blood. The data from each unit was subjected to analysis of variance to separate the day-to-day variability from the treatment variability. With the exception of one set of measurements, the results for the exposed and control samples were not statistically different at the three exposure levels used. The first set of measurements conducted at the SAR of approximately 2 W/kg did show a statistical difference at the $P = 0.05$ level, however, a repeat of the experiment did not confirm this difference. Results of both experiments are presented in Table 1.

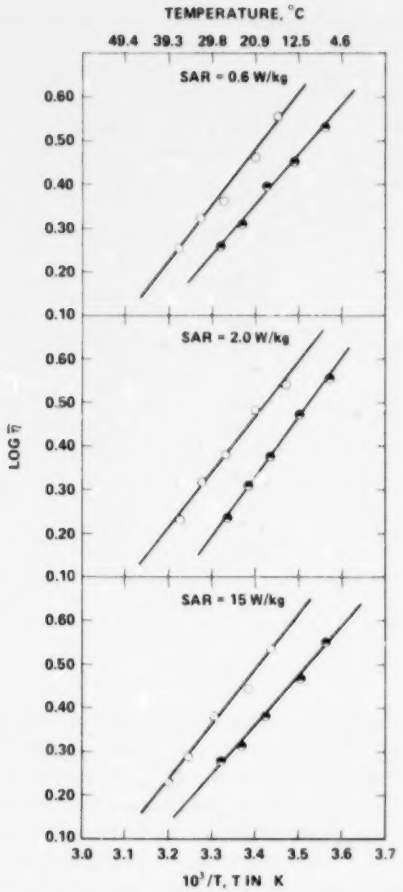

Fig. 1. Logarithm of the microviscosity vs inverse temperature. A representative plot from each exposure level is presented. The values for the control sample have been increased by 0.1 units on the abscissa for clarity. Open circles are exposed points and half-filled circles are control points.

The change in anisotropy, r, of DPH in red cell ghosts as a function of duration of exposure to 1.0-GHz radiation is shown in Figure 2. Measurements were obtained over a period of five hours at three exposure levels approximating those presented in Table 1. A repeated-measures analysis of variance was performed on the data for each exposure level. No statistically significant differences were found for the lower two SAR's; however, at the highest level, 14 W/kg, a statistically significant result (at $P = 0.02$) was found for both the treated vs control, and for the treatment-time interaction. The most probable explanation for this difference is that the temperatures of the exposed and control samples were the same (22.8 ± 0.5 °C) for the lower two SAR's, but we measured a 3.2 °C difference between the exposed and control samples at the highest SAR. The temperature difference was produced by the relatively high rate of energy absorption (14 W/kg), and could not

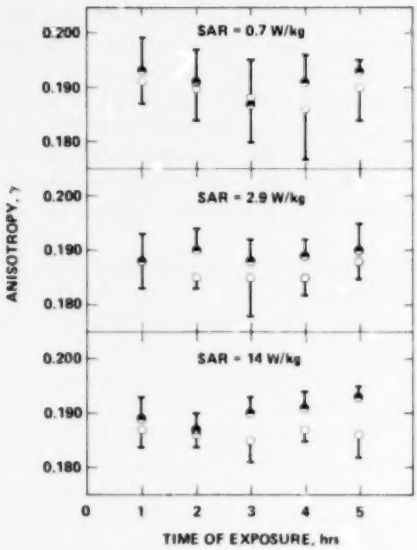

Fig. 2. The anisotropy is plotted as a function of time. The average and standard deviation are illustrated; n, the number of trials, is five for all points except at 5h and 0.7 W/kg where n is four. Open circles are exposed points and half-filled circles are control points.

be eliminated due to the constraints of the experiment. However, it is possible to correct the results for this temperature difference. Using equation (2) and the value of the slope from any of the Arrhenius plots in Figure 1, one may calculate that an increase of 3.2 °C corresponds to a decrease in the anisotropy, r, of 0.004. If this value is added to the results for the samples at an SAR of 14 W/kg, the values for the corresponding control and exposed samples are brought into agreement and the statistical significance disappears. Therefore, it appears that the temperature difference does account for the statistically significant effect found at 14 W/kg.

DISCUSSION

We have investigated the internal viscosity of the lipid of the human red cell membrane exposed to 1.0-GHz microwave radiation at selected temperatures between 15 °C and 40 °C at three SAR levels. These measurements have not shown any change in the membrane microviscosity as detected by our probe. Also, a series of experiments conducted as a function of exposure duration at 23 °C does not show a difference between exposed and control samples when the results are adjusted for the difference in temperature at the highest SAR level. The latter experiment is important to the interpretation of the Arrhenius-type plots, because the logistics of the experiment and the statistical variations encountered from day to day required that a single sample be exposed each day. Therefore serial measurements were made on a single sample to obtain a given curve. The anisotropy of DPH was found to be independent of the duration of exposure, and from this we may infer with confidence that the Arrhenius-type plots are not skewed by exposure duration.

The slope of a linear Arrhenius-type plot, such as we have obtained, is proportional to the activation energy for that process. In this case, the process under study is the motion of the fluorescent probe, DPH, under the influence of the viscous motion of the hydrocarbon tails of the membrane lipid. The fact that the slopes of the exposed and control samples are the same implies that a fundamental change in the motion of the hydrocarbon tails of the lipid molecules has not been induced by the microwave radiation. This conclusion applies to the mixed lipids of the red cell membrane which are presumably in the liquid-crystalline (as opposed to the gel-like) state, ie, above the phase transition temperature.

The activation energy calculated from the average of the slopes (control or exposed) in Table 1 is 5.6 kcal/mole. We calculated values of 4.4, 6.3 and 7.0 kcal/mole from the work of others using the same fluorescent probe, DPH [Weltzien et al, 1976; Aloni et al, 1975; Aloni et al, 1974]. The latter values were obtained from membranes prepared from freshly drawn blood. Kehry, et al [1977] have compared the microviscosity of red cell membranes from outdated human blood to that of freshly drawn neonatal blood using the probe perylene. They found the microviscosities for the two membranes indistinguishable. Their value of 2.56 poise at 25 °C is virtually identical to our results. Perylene and DPH are known to give similar results for the microviscosity of membranes in the liquid-crystalline state [Shinitzky and Barenholz, 1978]. Therefore, we conclude that our work is consistent with that of others, and for these measurements there is no distinguishable difference between fresh and outdated red cells.

Olcerst et al, [1980] have recently reported efflux of cations from rabbit red cells under microwave exposure, but only at certain transition temperatures. Their experiment is very different from the one we report here, but the results are relevant to each other. Olcerst et al suggested two general mechanisms to explain their results. One is based on the existence of two lipid phases separated laterally in the membrane. There is evidence for the gel-like and liquid-crystalline phase coexisting in E coli membranes [Linden et al, 1973], but to date there is no direct evidence for this phenomenon in red cells. The second hypothesis suggests that proteins in the membrane undergo conformational transitions at the key temperatures that are sensitive to microwave exposure.

The results presented here argue against the mechanism of lateral phase separation in the lipid portion of the membrane. DPH is a lipid seeking probe that distributes itself throughout the membrane regardless of composition and state [Shinitzky and Barenholz, 1978], and which has been shown to be very sensitive to a structural transition in the lipid. For example, by using DPH in liposomes that were a 50:50 mixture of dioleoylphosphatidyl choline and dipalmitoylphosphatidyl choline, Lentz et al, [1976] demonstrated a temperature profile that indicates transition points that are strikingly similar to the structural and functional transitions described by Linden et al using an E coli membrane of comparable composition. Therefore, it is probable that the ion efflux transitions observed by Olcerst et al are not related primarily to the state of the lipid in red cell membrane; rather, these phenomena are likely to be related to changes in membrane protein. The effect of the microwave radiation on ion efflux is likely to depend on the interaction of microwaves with membrane protein, perhaps in combination with limited amounts of associated water or membrane lipid.

The results of Tyazhelov et al [1979, 1980] also support this conclusion. Exposure to microwave radiation has changed the conductivity of phospholipid bilayers into which channel forming antibiotics have been incorporated. This change in flow of cations through the bilayer is consistent with the effect of Olcerst, et al [1980] if one postulates that the

cation transport proteins in the red cell membrane are sensitive to the imposed microwave field at the transition temperatures but not sensitive at other temperatures. A protein should be much more susceptible to disturbance by the microwave field as it is undergoing a structural transition in comparison to structurally stable states.

Although measurements of membrane microviscosity have not been performed on rabbit red cells, it is highly unlikely that the three transitions found by Olcerst, et al can be explained by lipid phase changes. Mammalian cell plasma membranes generally do not exhibit phase transitions due to the large fraction of cholesterol and mixture of lipids contained in them. Of the several mammalian red cell membranes that have been studied, only those from sheep exhibit a lipid transition, and that is a very broad one near 25 °C [Shinitzky and Barenholz, 1978].

This paper has presented evidence that the lipid interior of the red cell membrane does not undergo a fundamental change in properties during exposure to 1.0-GHz microwave radiation. The activation energy for the orientation of the probe, DPH, in the environment of the hydrocarbon tails of the membrane lipid, represented by the slopes of the Arrhenius-type plots, does not change. There is also no evidence for a cumulative effect of exposure over several hours. The results suggest that the phenomenon described by Olcerst et al [1980] is not related to lateral phase separation of membrane lipid.

ACKNOWLEDGMENTS

The authors are indebted to Dr. Claude M. Weil and Joseph S. Ali for assistance in maintaining the microwave exposure equipment and to Dr. William T. Joines for helpful discussions concerning the calculation of SAR. We would also like to acknowledge the assistance of Andrew Stead and Dr. L. Earl Gray in performing statistical analyses for this work. We are also indebted to Dr. Barry R. Lentz for providing very helpful information while we were establishing work with the membrane probe, DPH, in our laboratory.

This paper has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

REFERENCES

Allis JW, Blackman CF, Fromme ML, Benane SG (1977): Measurement of microwave radiation absorbed by biological systems. I. Analysis of heating and cooling data. Radio Sci 12(6S):1–8.

Aloni B, Shinitzky M, Livne A (1974): Dynamics of erythrocyte lipids in intact cells, in ghost membranes and in liposomes. Biochim Biophys Acta 348:438–441.

Aloni B, Shinitzky M, Moses S, Livne A (1975): Elevated microviscosity in membranes of erythrocytes affected by hereditary spherocytosis. Br J Haemotol 31:117–123.

Baranski S, Szmigielski S, Moneta J (1974): Effects of microwave irradiation in vitro on cell membrane permeability. In: "Biologic Effects and Health Hazards of Microwave Radiation." Warsaw: Polish Medical Publishers, pp 173–177.

Barnes FS, Hu CJ (1977): A model for some non-thermal effects of radio and microwave fields on biological materials. IEEE Trans Microwave Theory Tech MTT 25:742–746.

Dodge JT, Mitchell C, Hanahan DJ (1963): The preparation and chemical characteristics of hemoglobin-free ghosts of human erythrocytes. Arch Biochem Biophys 100:119–130.

Frolich H (1968): Böse condensation of strongly excited longitudinal electric modes. Phys Lett A 26:402.

Grodsky IT (1976): Neuronal membrane: A physical synthesis. Math Biosci 28:191–219.

Hamrick PE, Zinkle JC (1975): Exposure of rabbit erythrocytes to microwave radiation. Radiat Res 62:164–168.

Ismailov ESh (1971): The effect of microwaves on erythrocyte potassium and sodium ion permeability. Biol Nauky 14(3):58–60.

Joines WT, Dakermanji G, Seaman RL, Wachtel H (1976): Microwave power absorption by a rectangular-shaped conductive samples in stripline. IEEE Trans Microwave Theory Tech MTT 24:536–538.

Kehry M, Yguerabide J, Singer SJ (1977): Fluidity in the membranes of adult and neonatal human erythrocytes. Science 195:486–487.

Keilman F (1978): Nonthermal microwave resonances in living cells. In Arecchi FT, Bonifacio R, Scully MO (eds): "Coherence in Spectroscopy and Modern Physics," New York: Plenum Publishing Co, pp 347–360.

Lentz BR, Barenholz Y, Thompson TE (1976): Fluorescence depolarization studies of phase transitions and fluidity in phospholipid bilayers. I. Single component phosphatidylcholine bilayers. II. Two component phosphatidyl choline liposomes. Biochemistry 15:4521–4528, 4529–4537.

Linden CD, Wright KL, McConnell HM, Fox CF (1973): Lateral phase separation in membrane lipids and the mechanism of sugar transport in *Escherichia coli.* Proc Nat Acad Sci (USA) 70:2271–2275.

Liu LM, Nickless FG, Cleary SF (1979): Effects of microwave radiation on erythrocyte membranes. Radio Sci 14(6S):109–116.

Murphy JB, Kies MW (1960): Note on spectrophotometric determinations of proteins in dilute solution. Biochim Biophys Acta 45:382–384.

Olcerst RB, Belman S, Eisenbud M, Mumford WW, Rabinowitz JR (1980): The increased passive efflux of sodium and rubidium from rabbit erythrocytes by microwave radiation. Radiat Res 82:244–256.

Quinn PJ (1976): "The Molecular Biology of Cell Membranes." Baltimore: University Park Press.

Peterson DJ, Partlow LM, Gandhi OP (1979): An investigation of the thermal and athermal effects of microwave irradiation on erythrocytes. IEEE Trans Biomed Eng BME 26:428–435.

Seaman RL, Wachtel H (1978): Slow and rapid responses to CW and pulsed microwave radiation by Aplysia pacemakers. J Microwave Power 13:77–86.

Shinitzky M, Barenholz Y (1974): Dynamics of the hydrocarbon layer in liposomes of lecithin and sphingomyelin containing dicetylphosphate. J Biol Chem 249:2652–2657.

Shinitzky M, Barenholz Y (1978): Fluidity parameters of lipid regions determined by fluorescence polarization. Biochim Biophys Acta 515:367–394.

Shinitzky M, Dianoux A-C, Gitler C, Weber G (1971): Microviscosity and order in the hydrocarbon region of micelles and membranes determined with fluorescent probes. I. Synthetic micelles. Biochemistry 10:2106–2113.

Shtemler VM (1973): Certain principles governing the effects of microwaves on K^+ and Na^+ transport in human erythrocytes. In Gordon ZV (ed): "Biological Effects of Radiofrequency Electromagnetic Fields." Translated from Russian by Joint Publicatons Research Service, Arlington, VA, October 1974, JPRS–63321, pp 175–187.

Tyazhelov VV, Alekseyev SI, Grigor'ev PA (1979): Change in the conductivity of phospholipid membranes modified by alamethicin on exposure to high frequency electromagnetic fields. Biophysics 23:750–751 (translated from Biofizika 23.732–733, 1978).

Tyazhelov VV, Alekseyev SI, Faizona LK, Chertishchev VV (1980): Peculiarities of the microwave effect of gramicidin modified bilayers. Presented at the International Symposium on Electromagnetic Waves and Biology, Paris, France.

Weltzien HU, Arnold B, Kalkoff HG (1976): Quantitative studies on lysolecithin mediated hemolysis. Benzylated lysolecithin as a probe to study effects of temperature and red cell species on the hemolytic reaction. Biochim Biophys Acta 455:56–65.